APPRAISAL OF TYPHOID VACCINE IN EXPERIMENTALLY INFECTED HUMAN SUBJECTS

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The controversy regarding the efficacy of typhoid vaccine has existed since its introduction by Wright in 1896.[1] Two medical officers were given killed typhoid bacilli; subsequently, one was inoculated with viable *Salmonella typhosa*. It was assumed that the vaccine was protective since illness did not occur. Reports of innumerable uncontrolled trials since this introduction have failed to provide definitive answers. British troops were inoculated on a voluntary basis during the Boer war with inconclusive results. In 1908, Russell introduced typhoid immunization in the United States Army. None of the early field trials were subjected to proper controlled procedures; hence, the many claims of efficacy or lack of protection. In some reports, the incidence of cases and deaths was higher in the immunized than in the controls.

Following the classic studies of yellow fever in Cuba by Reed, one of his associates, James Carroll, collaborated with Edward Vedder in a typhoid immunization study.[2] They attempted to immunize by administering typhoid vaccine orally. The vaccine consisted of viable typhoid bacilli since their method of preparation by heat failed to completely inactivate. A number of severe cases of typhoid fever occurred in the volunteers which included one of the senior authors. The actual number of viable typhoid bacilli ingested is unknown.

An epidemiological study by Duncan, et al., in 1946, suggested strongly that the vaccine was efficacious.[3] Approximately 360 young women, including 181 SPARS who resided in a residential hotel for business women

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in Cleveland, Ohio, were involved in a localized epidemic. Most of 194 civilian women living in the hotel were not immunized in contrast to the SPARS. Orange juice, thought to be contaminated by a typhoid carrier, was regarded as the vehicle of infection, but this was not proven. There were seventeen cases of typhoid among 140 non-immunized women and one case in 211 previously given vaccine. The attack rate in the controls was 12.1 per cent. If the immunized had experienced the same incidence as the unimmunized, it was anticipated that twenty-five cases of typhoid should have occurred. Unfortunately, the source and dose of infection were unknown; on the basis of epidemiologic studies it was presumed that orange juice was the contaminating food.

In 1952, while working in collaboration with our associate, Joseph E. Smadel, we inaugurated quantitative studies designed to appraise the efficacy of typhoid vaccine. This program was under the general sponsorship of one of the Commissions of the Armed Forces Epidemiological Board during the chairmanship of Dr. Colin M. MacLeod. In these studies and those to follow, the strictest of standards which apply to the use of human subjects as volunteers were adhered to. Initially, two volunteers were exposed, one of whom was an immunologic virgin with respect to serologic negativity; the other showed a low titer of typhoid O and H agglutinins. Each volunteer ingested six million viable typhoid bacilli of the standard Ty2 strain. There was no clinical evidence of infection and serial cultures of the blood, bone marrow and bile showed no typhoid bacilli. There was no serologic response. These studies were postponed temporarily because of other vaccine trials.

In 1959, the study was resumed to systematically evaluate the typhoid vaccine and simultaneously to develop a better understanding of the pathogenesis of this ancient infection. Obviously, the first knowledge to be ascertained was the infectious dose of typhoid bacilli for man. The strain of typhoid bacilli employed (Quailes) was isolated from a typhoid carrier who had demonstrated its virulence recently by causing typhoid in the family. Our initial infecting doses were high in view of the failure to infect the two volunteers tested initially and studies in chimpanzees which showed that huge numbers of typhoid bacilli were required to infect this primate.[4] Typhoid bacilli previously isolated from the carrier and preserved by freezing were reconstituted, subcultured and placed in milk. Volunteers gargled and swallowed approximately one ounce of infected inoculum.

Infectious Dose, Clinical Illness, Complications

The infectious dose of typhoid bacilli for human subjects is shown in Table I. The ID_{50} dose is about ten million bacilli. Approximately 100

TABLE I

Number of Viable *S. typhosa*	Number with Typhoid / Number Challenged	Per cent
10^9	40/42	95
10^8	8/9	89
10^7	16/32	50
10^5	32/116	28
10^3	0/20	0

per cent of volunteers became ill with a 10^9 dose of bacilli. Typhoid fever provoked in volunteers was typical of naturally occurring disease with stepwise rise in fever which reached its height in about three days. Incubation periods varied and were inversely related to the size of the infective dose, i.e., the larger the dose, the shorter the incubation period. Range of incubation was three to twenty-six days.

Bacteriologic studies revealed that bacilli multiplied in the intestinal tract early. After ingestion stools yielded *S. typhosa* within twenty-four hours and remained positive during the incubation period. With a high infecting dose of 10^9 the stools invariably became positive for *S. typhosa* which was the precursor of active clinical infection. At the ID_{50} and ID_{25} dosage levels, although the stools yielded typhoid bacilli, clinical infection did not always occur.

Clinical manifestations were arrested uniformly by the giving of chloramphenicol usually on the third or fourth febrile day of illness. During the course of these studies, it was necessary to treat approximately 170 men for typhoid fever.

There have been no serious complications of any type. A few have shown a little occult bleeding and there have been no instances of perforation of the intestine. Several volunteers developed myalgia which abated. There were two instances of moderate hemolytic anemia and several developed temporary psychotic episodes. There have been no carriers.

Typhoid Vaccine Data

Three types of typhoid vaccine have been used although emphasis has been placed on two biologic preparations. Vaccines were developed at the Walter Reed Institutes of Medical Research. They consisted of a conventional type of phenol-heat inactivated vaccine which destroys most of the Vi antigen (designated L) and a vaccine consisting of typhoid bacilli inactivated with acetone which preserves much of the Vi antigen (designated vaccine K). A few volunteers were given a pure Vi type

vaccine made available by Dr. Maurice Landy of the National Institutes of Health. The program consisted of three doses, two with an interval of one week and the third dose given four weeks after the second. Vi vaccine was given in a single dose. The viable challenging infectious doses were given from three months to one year following the last immunizing dose. The typhoid vaccines, L and K, were identical with those used in field trials under the sponsorship of the World Health Organization in Yugoslavia, British Guiana, Poland and elsewhere.[5, 6] Times will not permit elaboration of the results of these field trials which were conducted under controlled conditions. These studies showed some degree of protection, particularly in children.

Vaccine Results

There was no protection in volunteers who were given an ID_{50} dose of infection of ten million *S. typhosa* or higher. Illness occurred in immunized and non-immunized subjects in spite of the height of the antibody titers prior to challenge. Indeed, some vaccinees became ill sooner than their non-vaccinated controls. There were the same number of relapses noted in either the vaccinated or the non-vaccinated. Moreover, illness was subsequently reproduced in some of the volunteers six months and one year after the primary infection using the same strain and the same dose of infecting bacilli. Hence, the prior clinical experience had not led to solid immunity.

At this point, we concluded justifiably that typhoid vaccine was not effective under the conditions of the study. An erroneous conclusion would have been made had we failed to continue the studies with a lower infecting dose. It was appreciated that perhaps too much was expected of the vaccine and that the infectious challenge of typhoid bacilli in nature might be lower; also, conditions in the host might be different and pertinent to susceptibility or resistance. It was necessary to study a large number of volunteers in order to determine the ID_{25} infectious dose. Note in Table I that this figure is approximately 100,000 typhoid bacilli. After this dose was determined, additional vaccinated and control human subjects were challenged.

Here the evidence became clear that the vaccines K and L offered some protection. At this challenge level, only about eight per cent of the vaccinees (K and L) became ill, or 92 per cent were protected. In contrast, 25 per cent of the non-vaccinated controls developed typhoid fever or 75 per cent did not become ill. A few volunteers given a purified Vi vaccine showed the same level of protection, no more or less than with vaccines K and L. Always the infection rate in the control subjects with 100,000 organisms was approximately 25 per cent, a fact which

permits these valid conclusions. Failure to test additional volunteers at the lower infectious dose would have made the trial much less significant.

The results of these volunteer studies show a correlation with the World Health Organization field trials. Apparently the typhoid vaccines are effective against a low challenge which might result from a water-borne exposure but offer no protection against heavier infectious doses subsequent to ingesting heavily contaminated foods which have incubated for indefinite periods.

Discussion

Obviously the vast majority of vaccinated or non-vaccinated human subjects failed to develop typhoid fever after ingesting pathogenic bacilli. The reasons for this paradox are pertinent to any understanding of pathogenesis as well as to those factors which constitute host resistance to typhoid fever. We believe that focus must be placed upon the ecology of the intestinal tract in order to understand susceptibility as well as defense against infection. The following findings and considerations are germane to this concept:

(1) Multiplication of bacteria does take place in the gastrointestinal tract and in the human trials just as many of these persons failed to develop typhoid fever as those who did.

(2) There was no serologic response in those vaccinated or unvaccinated volunteers who did not show evidence of circulatory invasion by typhoid bacilli. The effects of copro antibody, lysozymes and other factors require study.

(3) Established typhoid carriers harbor as many as sixty million or more *S. typhosa* per ml. of bile taken from the common bile duct and huge numbers in the intestinal tract. Yet in typhoid carriers there is no uniform serologic evidence of antigenic response such as the conventional antibodies, i.e., typhoid O, typhoid I or Vi. We suspect that it would be possible to bypass the gastrointestinal tract in carriers and provoke overt infection by giving an infectious dose of typhoid bacilli intravenously. Such studies might be accomplished in chimpanzees.

(4) Studies of the upper small intestinal mucosa obtained by biopsy during the incubation period, performed in collaboration with Dr. Helmuth Sprinz of WRAIR, have been helpful.[7] Inflammatory and granulomatous changes occurred in the epithelial lining of villi, crypt glands and in the tunica propria in relationship to the size of the infecting dose. More marked reactions occurred in those volunteers given the larger doses who subsequently developed typhoid fever. Those subjects given smaller doses showed minimal changes and often were not clinically ill.

(5) In 1956, Dr. C. Phillip Miller reported to this Association that the flora of the intestinal tract is related to susceptibility of mice to *Salmonella typhimurium* infection.[8] The giving of streptomycin to mice prior to administration of *S. typhimurium* markedly increased susceptibility. Streptomycin lowered the population of bacteroides in the intestinal tract and provoked a higher pH. This metabolic change favored infection. Japanese investigators reported increase in mouse susceptibility to salmonella infections by pre-treatment with antibiotics. Changes in population of lactobacilli in the intestinal tract were regarded as significant.

(6) Pre-treatment of volunteers with streptomycin or neomycin given orally increases susceptibility. As few as one thousand bacilli caused clinical illness. These studies are continuing.

(7) *S. typhosa* have the capacity to go underground or survive intracellularly in spite of the presence of circulating antibody or of antibiotics outside or within cells. The spheroplast concept deserves more attention.

These considerations force us to conclude that the environment and integrity of the intestinal tract are pertinent to our understanding of what constitutes resistance or susceptibility to typhoid fever.

Conclusion

The typhoid vaccines discussed appear to exert some protective value but the protection is of low order. We conclude that answers to some of the questions pertain to the ecology and state of resistance of the gastrointestinal tract. Focus should be directed to those cellular factors which constitute immunity rather than upon humoral factors per se. Further immunologic study of single cells and of penetrance and survival of typhoid bacilli within cells should provide important clues.

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DISCUSSION

PRESIDENT WATSON: These are very important observations, and I think we owe Dr. Woodward a debt of gratitude for being willing to give us this paper on such short notice.

DR. TIMOTHY TALBOT (Philadelphia): I am curious to know if you have any reason to suspect genetic aspects of this problem or if you have still got samples from these people that might be subjected to such analyses.

DR. EDWARD W. HOOK (New York): Dr. Woodward, I would like to ask if you have information on the number of typhoid bacilli required to produce typhoid fever under natural conditions. I believe there are some observations that might relate to this, and I would like to hear your comments about them.

DR. WOODWARD: Ed, do you mind quoting the ones that you have in mind?

DR. HOOK: I was thinking of the experiment carried out by Carroll and colleagues (*Military Med.* **124:** 342, 1959), but am uncertain of the number of organisms that were thought to be present in the preparations that were administered orally. I am also wondering about the number of viable bacilli in water responsible for outbreaks of typhoid fever. Do you have any information regarding this point?

DR. CLAUDE E. FORKNER (New York): Dr. Woodward, I believe about 1910 or '14, Wright made a very careful study and thought that the typhoid bacillus primarily invaded lymphoid tissue and that this was its primary site. Has that study stood up? How does one explain the early bacteremia in typhoid fever?

DR. A. MURRAY FISHER (Baltimore): You said that you have done some biopsies of the intestinal tract, I believe, and found that there is some hypertrophy or evidence of inflammation of the lymphoid tissue there. Have you been able to find any evidence of typhoid bacilli themselves in that tissue?

DR. FRANCIS MINOT RACKEMANN (Boston): When typhoid vaccine is given under the skin, the dose usually makes a local redness and swelling. Is there any evidence that the immunizing effect depends upon the size of that local reaction?

DR. JAMES A. CLIFTON (Iowa City): Dr. Woodward, I have forgotten the pathology of typhoid, but as I remember, the lesions are more prominent in the lower small intestine than the upper. Furthermore, bacterial growth in normal man is more luxuriant in the lower small intestine than in the upper small intestine. Do you know of any studies on growth of typhoid bacilli in mixed cultures such as one might take from the lower ileum?

DR. THOMAS W. MATTINGLY (Washington): I would like to state a personal observation and one in which I was not a volunteer. In 1928, I received a full series of Army vaccine before attending a summer medical ROTC camp. In 1931 I received a second series of vaccine while serving as a house officer in a civilian hospital where we had several patients with typhoid fever. In 1933, I developed typhoid fever. The source of the infection was not known but it was suspected that it was from oysters

from an infected oyster bed as I had eaten raw oysters about a week before I developed fever. There were similar cases reported in the area during that year. I entered the hospital about the fifth or sixth day of fever and a blood culture taken on the second day of hospitalization was positive for Salmonella Typhi.

I cite this experience as representing how active disease may occur in an individual who has received vaccine when subject to a massive infection such as the ingestion of raw infected oysters.

DR. COLIN M. MACLEOD (New York): I wonder, Ted, if you could throw any light on the nature of the protective antibody. You used three different vaccines in your volunteer studies: One of these was prepared so that the Vi antigen was destroyed by the method of preparation; in the second, the acetone-treated vaccine, Vi was preserved; the third vaccine consisted of Vi antigen itself in a purified form. However, despite these vaccine differences, you obtained the same amount of protection at the ID_{25} level with all three vaccines. How can this be explained?

DR. WOODWARD: Dr. Talbot, we have no data relative to genetic factors and susceptibility. ABO blood groupings have been given some consideration. Blood sera and other specimens are refrigerated which are to be analyzed for their biochemical and immunological composition which may shed some light.

To answer Dr. Hook: There have been instances of laboratory infections and small outbreaks when the source was known. In spite of this, the exact number of infecting viable typhoid bacilli is unknown. The Carroll-Vedder experience is perhaps the best example involving a number of persons, yet the exact number of organisms in the infectious inoculum was not known.

The outbreak caused by ingesting contaminated orange juice does not provide specific figures. A typhoid carrier was said to have contaminated orange juice which was the source of infection incriminated on epidemiologic evidence. Typhoid bacilli were not isolated from orange juice ingested by the young women. It was shown subsequently that orange juice is a suitable medium to support the growth of *S. typhosa*. There are other examples, Dr. Hook, but I cannot cite the exact numbers of typhoid bacilli involved. Your interpretation is probably quite correct that smaller numbers of typhoid bacilli are involved in nature's experiments.

Dr. Forkner's question pertains to involvement of lymphoid tissue. Years ago, Dr. Ernest Goodpasture demonstrated the presence of organisms thought to be typhoid bacilli in the cytoplasm of plasma cells. He performed an autopsy on a young typhoid patient immediately after death. With persistance, he demonstrated intracellular organisms. The early writings of Eberth mention the intracytoplasmic habitat. Perhaps typhoid bacilli assume stage variations in plasma and other cells which makes their visualization difficult.

We performed serial studies of all types in the infected volunteers and were surprised initially to note bacteremia early. This apparently occurs soon after successful invasion and the reticuloendothelial system must effectively clear many bacilli. Perhaps they escape from such phagocytic cells to contribute to the bacteremic phase later.

When Dr. Hornick by-passed the pharyngeal mucosa by instilling typhoid bacilli in the stomach, he noted that mild pharyngitis occurred. This suggested nonspecific inflammatory reaction involving pharyngeal lymphoid tissues although it could be argued that typhoid bacilli reached the area in large numbers via the vascular system. Quantitative bacteriologic studies in chimpanzees infected experimentally show relatively few bacteria in the tonsil on about the fourth day of infection.

The early bacteremia apparently follows the successful invasion of the intestinal barrier. Usually it signifies clinical illness although a few patients with bacteremia have been asymptomatic.

Dr. Rackemann, I am not aware of the relationship of dose size and the reaction in the intradermal tissues. In a previously immunized person 0.1 ml. of vaccine can produce an antibody response equal to 1.0 ml. of vaccine given subcutaneously. Subjects repeatedly given 0.1 ml. of vaccine intradermally develop progressively severe local reactions of erythema and edema.

Dr. Clifton, I know of no reliable data with respect to the relationship of mixed cultures taken from the lower intestinal tract and the growth of typhoid bacilli. It was mentioned that members of the species Bacteroides, Lactobacilli and E. coli-Proteus group exhibit an inhibitory in-vivo action, probably by changes in pH within the intestine. You are quite correct that the dominant changes in typhoid fever are in the lower small intestinal tract, particularly the terminal ileum. Our biopsy specimens were taken from the upper small bowel and showed some inflammatory changes during the early stages which suggest that invasion may occur in this area.

To answer Dr. Murray Fisher: No typhoid bacilli were observed microscopically in the mild inflammatory lesions of the upper gastrointestinal tract. However, we must try to localize antigens in these intestinal cells by immunochemical methods or by electron microscopy. This is planned.

Dr. Mattingly's comments are appreciated and although he may now boast of some immunity, he should not break too many rules of good preventive medicine.

Dr. MacLeod put his finger on the very important problem of the protective antibody. We believe that the human trials are significant in showing that a vaccine with a small amount of Vi antigen (phenol inactivated) shows about the same level of protection as a purified Vi antigen. A small amount of Vi antigen is present in the phenol inactivated vaccine. The Vi antigen per se in probably not responsible for provoking immunity yet we lack knowledge of what constitutes the protective antibody. Rather, we feel that focus must be on cellular immunity and events within the tissue cell, particularly of the intestine, should be clarified.

Thank you for these questions. (Applause)